BioMetals 1995, 8, 3–11

MINI-REVIEW

Current aspects in metal genotoxicity

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Received 7 March 1994; accepted for publication 15 June 1994

While carcinogenic metal ions are mostly non-mutagenic in bacteria, different types of cellular damage have been observed in mammalian cells, which may account for their carcinogenic potential. Two modes of action seem to be predominant: the induction of oxidative DNA damage, best established for chromium compounds, and the interaction with DNA repair processes, leading to an enhancement of genotoxicity in combination with a variety of DNA damaging agents. In the case of Cd(II), Ni(II), Co(II), Pb(II) and As(III), DNA repair processes are disturbed at low, non-cytotoxic concentrations of the respective metal compounds. Even though different steps in DNA repair are affected by the diverse metals, one common mechanism might be the competition with essential metal ions.

Keywords: metal compounds, DNA damage, mutagenicity, DNA repair, interactions

Introduction

Compounds of chromium, nickel, cadmium, lead, cobalt and arsenic are carcinogenic to humans and/or to experimental animals (Table 1). Nevertheless, the mechanisms involved in tumor formation are still unclear, since most metal compounds revealed no genotoxic potential in bacteria and rather weak mutagenic responses in mammalian cells in culture. However, when performing more detailed investigations and including diverse endpoints of cellular damage, cultured mammalian cells in combination with *in vitro* studies have proven to be valuable tools in elucidating mechanisms of action which may be related to the carcinogenic potential of metals (Table 1).

Several factors contribute to the weak genotoxic activity in intact cells. In general, the cellular toxicity varies considerably not only between compounds of the diverse metals, but also between different species of one metal, due to differences in bioavailability. The uptake of metal ions depends strongly on factors like oxidation state and solubility; while some metal species like Cr(VI) are able to enter intact cells readily, others like Ni(II) or Co(II) are taken up only slowly and require long incubation times. Furthermore, some metal ions exert higher affinities to amino acids as compared to nucleotides, leading rather to an inactivation of proteins involved in DNA replication, DNA transcription and DNA repair processes. Finally, compounds of Ni(II) have been shown to damage distinct chromosome regions preferentially, while the overall DNA damage is comparatively low.

Table 1. Evidence for the carcinogenicity of some metal compounds evaluated by the IARC[a]

Metal	Humans[b]	Animals[b]	Overall evaluation[c]
Arsenic	S	L	group 1
Cadmium and cadmium compounds	S	S	group 1
Cr(VI) compounds	S	S[d]	group 1
Cobalt and cobalt compounds	I	S[e]	group 2B
Lead	I	S[f]	group 2B
Nickel and nickel compounds	S	S[g]	group 1

[a]IARC (1980, 1987, 1990, 1991, 1993).
[b]S, sufficient evidence; L, limited evidence; I, inadequate evidence.
[c]Group 1: carcinogenic to humans; Group 2B: possibly carcinogenic to humans.
[d]Calcium chromate, lead chromate, strontium chromate, zinc chromate.
[e]Cobalt metal powder, Co(II) oxide.
[f]Inorganic lead compounds.
[g]Nickel monoxides, crystalline nickel sulfides, nickel hydroxides.

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When taking these special features into account and employing appropriate incubation conditions, toxic metal ions lead to diverse types of damage to different cellular constituents, including DNA, when applying *in vitro* systems, intact cells in culture or animal studies, mainly due to competition with essential metal ions and the ability of transition metals to catalyze the formation of free radical species. Regarding the genotoxicity, two mechanisms seem to be predominant: the generation of oxidative DNA damage, and the interference with DNA repair and DNA replication processes. This review briefly summarizes the present knowledge on metal genotoxicity and will discuss the potential significance of the findings for the carcinogenicity of the respective metals.

DNA damage and mutagenicity: potential role of reactive oxygen species in metal-induced genotoxicity

Due to the redox chemistry of transition metals and the ability to activate oxygen species in the course of redox reactions, one frequently discussed mechanism in metal-induced genotoxicity is the formation of oxidative DNA damage. In this context, compounds of Cr(VI), Fe(II)/Fe(III), Ni(II) and Co(II) have been shown to damage DNA *in vitro*, e.g. in the presence of H_2O_2. Furthermore, oxidative DNA damage might occur via the inactivation of cellular defense systems (for review, see Kasprzak 1991). However, the most reactive oxygen species, like hydroxyl radicals, are rather short-lived and in intact cells defense systems exist to detoxify free oxygen radicals. Therefore, the potential relevance of oxidative damage for intact cells depends on uptake, complex ligands, intracellular distribution and reductants, all of which affect the concentrations of metal ions available for redox reactions close to critical targets like the DNA.

Table 2. Genotoxicity of metal compounds in mammalian cells[a]

Metal compound	DNA damage	Mutagenicity[b]
Cr(VI)	DNA strand breaks, chromosomal aberrations	+
Ni(II)	DNA strand breaks[c], chromosomal aberrations	(+)
Co(II)	DNA strand breaks, chromosomal aberrations	+
Fe(III)-NTA	DNA strand breaks, sister-chromatid exchanges	+
Cd(II)	DNA strand breaks, chromosomal aberrations[d]	(+)
As(III)	chromosomal aberrations	–
Pb(II)	DNA strand breaks[d]	(+)

[a]For references, see text.
[b]+, positive; (+), weak effect and/or restricted to cytotoxic concentrations.
[c]Controversial results.
[d]Restricted to highly cytotoxic concentrations.

Chromium

For chromium compounds, the induction of DNA damage and the mutagenic potentials are consistent with their carcinogenic action. Strongest effects are observed with Cr(VI), which is readily taken up by cells in culture, followed by the intracellular reduction to Cr(III) and the induction of DNA strand breaks, DNA protein cross-links and 8-hydroxyguanine, a premutagenic DNA lesion mediated by oxygen free radical attack on the DNA. While the induction of these types of DNA damage is well established, current research is focussing on the identification of the ultimate genotoxic species responsible for the induction of the respective DNA lesions. Possible candidates are comparatively stable Cr(V) intermediates, Cr(IV), Cr(III) and/or reactive radical species generated during the reduction process (for review, see Cohen *et al.* 1993).

Nickel

Compounds of nickel are carcinogens in humans and experimental animals. The carcinogenic potency is highest for particulate nickel with intermediate water solubility; by applying mammalian cells in culture, this observation could be attributed to differences in bioavailability, since certain particulate nickel compounds are phagocytosed, yielding high amounts of nickel ions in the nucleus. Regarding DNA damage in mammalian cells in culture, the induction of DNA strand breaks is controversial, but DNA–protein cross-links as well as chromosomal aberrations are induced in mainly heterochromatic regions (for review, see IARC, 1990). In this context, Ni(II) has been shown to induce deletions in human and Chinese hamster embryo cells, leading to a loss of X-linked genetic activity associated with cellular senescence (Wang *et al.* 1992). As one possible mechanism for DNA damage induction, the production of reactive oxygen species has been suggested, presumably by Fenton-type reactions. In support of this model, oxidative DNA damage induced by Ni(II) has been observed in isolated DNA and chromatin in the presence of H_2O_2 (for review, see Kasprzak 1991). Recently, Ni(II) complexed to the tripeptide Gly–Gly–His in combination with H_2O_2 has been shown to induce mutations *in vitro* on single-stranded M13mp2 DNA as scored by a forward mutation assay in *Escherichia coli*, including tandem double CC → TT mutations typical for damage by oxygen free radicals (Tkeshelashvili *et al.* 1993). Evidence for the generation of oxidative DNA damage by Ni(II) has also been obtained in experimental animals. An elevated level of 8-hydroxyguanine was induced in rat kidney cells after a single i.p. injection of nickel acetate (Kasprzak *et al.* 1990). Furthermore, a GGT → GTT transversion was the predominant mutation in the K-*ras* oncogene in renal sarcomas of male Fisher rats induced by nickel subsulfide in combination with Fe^0 particles (Higinbotham *et al.* 1992). However, data supporting the induction of oxidative DNA damage in mammalian cells in culture are still missing. Since Ni(II) is mostly non-mutagenic in bacterial test systems and only weakly mutagenic in mammalian cell lines (IARC 1990), the carcinogenic potential of nickel

compounds is not readily explained by its mutagenic action.

Cobalt

Even though cobalt is an essential trace element, it induces tumors in experimental animals and has been classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC 1991). Carcinogenic and mutagenic effects of cobalt compounds have been reviewed recently (Léonard & Lauwerys 1990, Beyersmann & Hartwig 1992). Cobalt was mostly non-mutagenic in bacterial test systems (for review, see Beyersmann & Hartwig 1992); however, this might be rather due to an inhibition of the mutagenic response by medium components, since Pagano & Zeiger (1992) found Co(II) to be mutagenic in *Salmonella typhimurium* strain TA 97 when preincubated in distilled water or HEPES buffer, but not in medium containing phosphates, citrates and Mg(II).

In mammalian cells in culture, Co(II) induced DNA strand breaks in different cell types as well as DNA protein cross-links. Furthermore, Co(II) is clastogenic, inducing chromosomal aberrations, micronuclei and sister chromatid exchanges; all of these effects were observed at basically non-cytotoxic concentrations (for review, see Beyersmann & Hartwig 1992). Concerning the mutagenic potential, Co(II) is weakly mutagenic at the *hprt* locus after 24 h of incubation (Hartwig *et al.* 1990a, 1991) but non-mutagenic at the *tk* locus in mouse lymphoma L5178Y/TK$^{+/-}$ cells after 3 h treatment (Amacher & Paillet 1980), presumably due to a lack of bioavailability in the latter study. As one possible mechanism of genotoxicity, the generation of reactive oxygen species has been proposed in several studies. In the presence of hydrogen peroxide, Co(II) catalyzed the degradation of deoxyribose, induced DNA cleavage in ^{32}P-5′-end-labeled DNA and induced DNA base damage typical for hydroxyl radical attack in nuclear chromatin isolated from human K562 cells. The formation of hydroxyl radicals but also superoxide anions was also suggested from experiments applying free radical scavengers (for review, see Beyersmann & Hartwig 1992). Therefore, the generation of oxygen free radicals by Co(II) and H_2O_2 with subsequent DNA damage is well established for *in vitro* systems; however, the role of these radical species in cobalt genotoxicity in intact cells in culture has not been elucidated yet.

Iron

Iron is an essential element and involved in many cellular reactions. However, due to its ability to catalyze Fenton-type reactions, the production of highly reactive oxygen species like hydroxyl radicals and superoxide anions has been observed by different approaches in cell free systems. In intact cells in culture, cellular damage is prevented to a high degree due to the binding to storage proteins like ferritin and the inactivation of reactive oxygen species by cellular defense systems (for review, see Halliwell & Gutteridge 1990). For example, ferric citrate caused a dose- and time-dependent induction of single- and double-strand breaks in isolated supercoiled plasmid DNA (Toyokumi & Sagripanti 1993), while neither lipid peroxidation, DNA strand breaks nor sister chromatid exchanges were observed in intact V79 cells (Hartwig *et al.* 1993). However, the effectiveness of cellular defense systems depends on the actual iron complex administered. When investigating ferric nitrilotriacetate (Fe-NTA), this complex caused renal carcinomas in rats (Ebina *et al.* 1986) as well as elevated levels of 8-hydroxyguanine in DNA in the same organ (Umemura *et al.* 1990). Furthermore, the induction of mutations at the *hprt* locus (Nakatsuka *et al.* 1990) as well as the induction of lipid peroxidation, DNA strand breaks and sister chromatid exchanges were shown in V79 Chinese hamster cells (Hartwig *et al.* 1993).

Arsenic

Chronic exposure to arsenic compounds leads to skin and lung cancer in exposed humans, while animal studies failed to detect a carcinogenic potential (for review, see Goldman & Dacre 1991, Bates *et al.* 1992). Regarding the genotoxicity, arsenic is not mutagenic in bacterial test systems nor in mammalian cells. In contrast, its clastogenic potential to produce mainly chromatid type chromosomal aberrations and sister chromatid exchanges is well documented with As(III) being the more potent form compared with As(V) (for review, see Jacobson-Kram & Montalbano 1985).

Cadmium

Cadmium is carcinogenic to humans and experimental animals, independent of the compound tested (Oldiges *et al.* 1984, 1989; Stayner *et al.* 1992). However, the underlying mechanisms are still puzzling. Cd(II) is mostly nonmutagenic in bacterial test systems and only weakly mutagenic in mammalian cells in culture. Concerning the induction of DNA damage, Cd(II) has been shown to induce DNA strand breaks and chromosomal aberrations in V79 Chinese hamster cells; experiments applying free radical scavengers supported the involvement of oxygen free radicals in this process, which in turn might be mediated by a decrease in intracellular glutathione content induced by Cd(II) on similar conditions (for review, see Hartwig 1994). However, the relevance of these findings for the carcinogenic action of cadmium seems questionable, since—except for the weak mutagenic action—both clastogenicity and DNA damage were restricted to highly cytotoxic concentrations of Cd(II).

Lead

Based on the induction of mainly renal tumors in rats and mice after different routes of delivery, lead compounds are classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC 1980, 1987). In contrast to animal studies, epidemiological evidence for a carcinogenic potential and the induction of

chromosomal aberrations in exposed people are still controversial (Gebhart & Rossman 1991). Regarding mammalian cells in culture, the mutagenic potential is weak and restricted to long incubation times (5 days) or high, cytotoxic concentrations. Similarly, no DNA damage, such as DNA strand breaks, DNA protein cross-links or sister chromatid exchanges, were detected (Zelikoff *et al.* 1988), except for one study, where lead acetate and lead nitrate induced DNA strand breaks at toxic doses as determined by nick translation (Roy & Rossman 1992).

Modulation of genotoxicity by metal compounds: potential role of DNA repair inhibition

It was first demonstrated in *E. coli* that arsenite—even though not mutagenic itself—increased the mutation frequency when combined with UV light (Rossman 1981). Later on, similar comutagenic effects towards UV light were observed for Co(II) and Mn(II) (Rossman & Molina 1986) as well as for Ni(II) (Dubins & LaVelle 1986) and Cd(II) in combination with alkylating agents (Mandel & Ryser 1984), suggesting that an inhibition of DNA repair processes may be the predominant mechanism in metal-induced genotoxicity. Even though these results obtained in bacterial test systems are not directly transferable to eukaryotic cells due to considerable differences in cellular repair systems, most carcinogenic metal compounds have been shown to increase the cytotoxicity, mutagenicity and clastogenicity when combined with different types of DNA damaging agents in mammalian cells (Table 3). In the case of As(III), Ni(II), Cd(II), Co(II) and Pb(II) these effects were found in a close relationship to interactions with DNA repair processes.

Arsenic

There are several studies indicating that the interaction with DNA repair processes might be the predominant mechanism in arsenic-induced genotoxicity as compared with direct DNA damage. As stated above, arsenic enhanced the mutagenicity of UV light in *E. coli*, but also in mammalian cells it has been shown to enhance the cytotoxicity, mutagenicity and clastogenicity in combination with UV light, X-rays, alkylating agents as well as DNA cross-linking compounds. A link between the

Table 3. Enhancement of cytotoxicity and genotoxicity by metal compounds in combination with different DNA damaging agents in mammalian cells[a]

Metal compound	DNA damaging agent	Enhancement of	Cell line	Reference[b]
As(III)	UVC	mutations (*hprt*)	V79; CHO	1–3
		cytotoxicity	human fibroblasts	2
		CA	CHO	4
	MMS, MNU	mutations (*hprt*)	CHO, V79	5, 6
	X-rays	CA	human lymphocytes	7
	cis-Pt	CA, cytotoxicity	human fibroblasts, CHO	8
	8-MOP + UVA	CA	CHO	
Cd(II)	UVC	mutations (*hprt*)	V79	9
	benzo[*a*]pyrene	cell transformations	SHE	10
	MMC,4NQO, *cis*-Pt	CA	CHO	11
Ni(II)	UVC	cytotoxicity, mutations (*hprt*), SCEs	V79	12, 13
	benzo[*a*]pyrene	mutations (Na^+/K^+-ATPase), cell transformations	SHE	14
	cis-Pt, *trans*-Pt, MMC	cytotoxicity	V79	15–17
Co(II)	UVC	cytotoxicity, mutations (*hprt*)	V79	18
Pb(II)	UVC	mutations (*hprt*; *E. coli gpt*), SCEs	V79	19, 20
	MNNG	mutations (*E. coli gpt*)	V79	20

[a]Abbreviations: CA, Chromosomal aberrations; CHO, Chinese hamster ovary; SHE, syrian hamster embryo; 8-MOP, 8-methoxypsolaren; MMC, Mitomycin C; MMS, methyl methanesulfonate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MNU, methyl nitrosourea; 4NQO, 4-nitroquinoline 1-oxide; *cis*-Pt, *cis*-diamminedichloroplatinum(II); *trans*-Pt, *trans*-diamminedichloroplatinum(II); SCE, sister chromatid exchange; UVA, UV 360 nm; UVC, UV 254 nm.

[b]References: 1, Lee *et al.* (1985); 2, Okui & Fujiwara (1986); 3, Yang *et al.* (1992); 4, Huang *et al.* (1992); 5, Lee *et al.* (1986a); 6, Li & Rossman (1989a, b); 7, Jha *et al.* (1993); 8, Lee *et al.* (1986b); 9, Hartwig & Beyersmann (1989b); 10, Rivedal & Sanner (1981); 11, Yamada *et al.* (1993); 12, Hartwig & Beyersmann (1989a); 13, Christie (1989); 14, Rivedal & Sanner (1980); 15, Hartwig *et al.* (1989a); 16, Hartwig *et al.* (1994a); 17, Krueger *et al.*, unpublished; 18, Hartwig *et al.* (1991); 19, Hartwig *et al.* (1990b); 20, Roy & Rossman (1992).

enhancing effects and the inhibition of DNA repair processes has been documented by Okui & Fujiwara (1986): while both As(III) and—at higher concentrations—As(V) increased the sensitivity of normal human fibroblasts towards UV light, no effect was seen in the repair deficient cell line Xeroderma pigmentosum group A. In the same study, arsenic in both oxidation states reduced the unscheduled DNA synthesis (UDS) and the removal of cyclobutane pyrimidine dimers after UV irradiation; the latter effect was also confirmed by Snyder *et al.* (1989) in HeLa cells. The interaction of As(III) with the DNA excision repair pathway has been further characterized by Li & Rossman (1989b) when applying nuclear extracts from arsenic treated V79 cells: both ligase I and—more pronounced—ligase II, which are involved in DNA replication and DNA repair processes, respectively, exerted a reduced activity compared to control cell extracts when annealing synthetic oligonucleotides. An inhibition of the ligation step has also been supported by Lee-Chen *et al.* (1992), who observed a delayed rejoining of repair-mediated DNA strand breaks after UV irradiation in CHO cells. Since DNA lesions are not only induced by exogeneously administered agents but—mainly oxidative—DNA damage is also generated endogeneously, an inhibition of the ligase activity could also explain the clastogenic activity by arsenic itself. As possible mechanism of ligase inhibition the inactivation of essential sulfhydryl groups has been discussed (Li & Rossman 1989b). However, the analysis of the mutational spectrum induced by UV light in the absence and presence of sodium arsenite suggests that not only the inhibition of DNA excision repair, but also an interference with the replication fidelity in the mutation fixation process contributes to the comutagenic activity of arsenite (Yang *et al.* 1992).

Nickel

Although not mutagenic itself, Ni(II) enhanced the mutagenicity of methyl methanesulfonate in *E. coli* (Dubins & LaVelle 1986). Similar effects have been observed in mammalian cells: Ni(II) enhanced the number of mutations and cell transformations induced by benzo[*a*]pyrene, the UV-induced cytotoxicity, mutagenicity and sister chromatid exchanges, and reduced the colony forming ability in combination with different cytostatic agents, which predominantly produce diverse types of DNA–DNA cross-links (Table 3). As one possible mechanism of the enhancing effects described, Ni(II) has been shown to interfere with the repair of UV- and X-ray induced DNA damage. In HeLa cells, no removal of UV-induced thymine-thymine dimers was observed in the presence of Ni(II) (Snyder *et al.* 1989). In our laboratory, different attempts have been made to further identify which step in nucleotide excision repair is affected. First of all, we investigated the repair of cyclobutane pyrimidine dimers by the determination of T4 endonuclease V-sensitive sites in combination with the alkaline unwinding technique after irradiation with very low, biologically relevant doses of UVC light. No loss of enzyme sensitive sites was detected within 5 h after irradiation in the presence of Ni(II), indicating that no substantial number of incisions occur at the sites of these lesions. In addition, the alkaline unwinding as well as the nucleoid sedimentation technique were applied to follow the kinetics of incisions and ligations after UV irradiation. These data demonstrate that the number of incisions is significantly reduced in HeLa cells treated with non-cytotoxic concentrations of Ni(II), suggesting an interference with the damage recognition/incision step in nucleotide excision repair. However, once incisions are made, the ligation of repair patches is delayed also compared with control cells, indicating that postincision events like the polymerization/ligation step are affected as well (Hartwig *et al.* 1994b). An inhibition of the ligation step was also observed by Lee-Chen *et al.* (1993) in CHO cells. Regarding the interactions with the repair of X-ray induced DNA damage, an impaired ligation of DNA strand breaks was reported for HeLa (Snyder *et al.* 1989) and CHO cells (Christie 1989). As a possible mechanism of repair inhibition, the competition by Ni(II) with Mg(II) seems to play an important role. The interference with the repair of UV-induced DNA damage is partly reversible by the subsequent addition of Mg(II) (Hartwig *et al.* 1994b). In summary, the inhibition of DNA repair by Ni(II) is well documented and may well explain its enhancing effects in combination with other DNA damaging agents. The diversity of DNA damaging agents affected by Ni(II) suggests that excision repair processes are affected in general; the fact that both the damage enhancing effects and the repair inhibition occur at non-cytotoxic concentrations indicate their potential relevance for the genotoxicity and perhaps carcinogenicity of nickel compounds.

Cobalt

Cobalt has been shown to modulate the genotoxicity induced by other DNA damaging agents in bacterial and in mammalian test systems. Interestingly, opposite results were obtained in both types of organisms. In *E. coli*, Co(II) decreased the mutation frequency induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, UV light or X-rays (Kada & Kanematsu 1978, Kada *et al.* 1986, Leitão *et al.* 1993) and lowered the number of spontaneous mutations in a *Bacillus subtilis* mutator strain (Inoue *et al.* 1981). In contrast, in mammalian cells in culture, Co(II) increased the frequency of UV-induced mutations and sister chromatid exchanges (Hartwig *et al.* 1991), but decreased the mutation frequency and enhanced the cytotoxicity in combination with γ-rays (Yokoiyama *et al.* 1990). As possible reason for the enhancing effects in combination with UV light, Co(II) has been shown to completely block the removal of UV-induced pyrimidine dimers at non-cytotoxic concentrations. When applying the nucleoid sedimentation and the alkaline unwinding technique to follow the induction and closure of DNA strand breaks occurring during early repair processes, Co(II) caused an accumulation of DNA strand breaks,

indicating that the polymerization or ligation step is inhibited (Hartwig *et al.* 1991, Kasten *et al.*, in preparation). In contrast, no inhibition of DNA strand break resealing after X-irradiation was observed in HeLa cells (Snyder *et al.* 1989), suggesting that Co(II) acts rather specifically on the nucleotide excision repair pathway. The antimutagenic effects observed in bacterial test systems might be due to an inhibition of DNA repair systems as well. Mutation induction in procaryotic cells following replication-blocking DNA lesions is mediated by the so-called SOS response, a process leading to an enhanced synthesis of repair proteins, but also to a decreased fidelity of polymerase III, thereby enabling it to synthesize DNA opposite DNA lesions and increasing the error frequency (Livnch 1986). This process depends on protein synthesis, which has recently been shown to be blocked by Co(II) (Leitão *et al.* 1993). Therefore, inhibition of error-prone repair may lead to antimutagenesis, while an interference with the largely error-free excision repair process in eucaryotes enhances the genotoxicity in combination with other DNA damaging agents. These discrepancies between procaryotic and eucaryotic test systems demonstrate that data obtained from bacterial test systems cannot readily be transferred to mammalian cells.

Cadmium

In bacterial test systems, Cd(II) is comutagenic in combination with methyl nitrosourea (MNU) (Mandel & Ryser 1984, 1987, Takahashi *et al.* 1988), presumably due to the inactivation of the O^6-methylguanine-DNA-methyl transferase (MGTase), a repair protein responsible for the removal of O^6-methylguanine, a premutagenic DNA lesion induced by alkylating agents. A mechanism proposed for this inactivation is the binding of Cd(II) to methyl group acceptor sites of the O^6-methyltransferase, essential for transcriptional activation of its own biosynthesis (Takahashi *et al.* 1992). In mammalian cells we observed an enhanced frequency of UV-induced mutations in Cd(II) treated cells (Hartwig & Beyersmann 1989a) as well as an increase in UV-induced cytotoxicity in various different rodent and human cell lines, which was no longer detectable by simultaneous incubation with Zn(II) and Cd(II). This enhancement of cytotoxicity could be attributed to an inhibition of DNA repair after UV-irradiation, since no enhancing effect was observed in repair deficient human fibroblasts Xeroderma pigmentosum complementation group A (Hartwig & Beyersmann, in preparation). Similar effects were observed by Yamada *et al.* (1993): When investigating the clastogenic activity of Cd(II) alone and in combination with different DNA damaging agents in Chinese hamster ovary cells, no increase in chromosome aberrations was observed with cadmium alone; however, it increased the frequency of chromatid aberrations induced by mitomycin C, 4-nitroquinoline 1-oxide, cisplatin and methyl methanesulfonate, but not bleomycin or actinomycin D. When applying Xeroderma pigmentosum cells complementation group A, no co-clastogenic effect was observed with 4-nitroquinoline 1-oxide, but still—although weaker—with mitomycin C. These findings might be highly relevant for the carcinogenic action of Cd(II), since—in contrast to the direct DNA damage described above—these diverse enhancing effects were observed at completely non-cytotoxic concentrations of the metal. An inhibition of DNA repair has also been investigated directly. In HeLa cells, Cd(II) blocked the removal of UV-induced pyrimidine dimers (Snyder *et al.* 1989). Following UV irradiation in human fibroblasts, Cd(II) caused a reduction in unscheduled DNA synthesis (UDS) as well as an accumulation of DNA strand breaks as determined by alkaline elution, indicating an inhibition of the polymerization step in nucleotide excision repair (Nocentini *et al.* 1987).

Lead

In contrast to the only limited evidence for DNA damage and mutagenicity in mammalian cells, lead compounds show more pronounced enhancing effects in combination with UV light and alkylating agents (Table 3). Again, an interference with DNA repair systems seems to be the likely explanation: when investigating repair processes after UV-irradiation by the nucleoid sedimentation technique, lead ions caused an accumulation of DNA strand breaks, indicating an inhibition of the polymerization/ligation step in excision repair (Hartwig *et al.* 1990b).

DNA repair inhibition: mechanistic aspects

There is increasing evidence that the inhibition of DNA repair processes provides one important mechanism in the genotoxicity of carcinogenic metal compounds. Regarding the nucleotide excision repair pathway in eucaryotic cells, the major repair system involved in the removal of bulky DNA damage induced by a variety of chemical and physical agents, repair events depend on complex DNA–protein interactions. Studies with repair deficient human and rodent cell lines identified at least eight to 10 proteins involved in the damage recognition/incision step, followed by repair polymerization and ligation of repair patches. In principle, the inhibition of DNA repair processes by carcinogenic metal ions may be due to structural changes of the DNA or modifications of repair proteins. Even though the experiments carried out so far demonstrate that toxic metal ions interfere with different steps in excision repair (Figure 1), the competition with essential metal ions serving as cofactors required at different steps in excision repair might play an important role. Regarding Ni(II), the partial reversibility of repair inhibition by the addition of Mg(II) has been demonstrated (see above). This competition with magnesium may affect several steps in the repair process: (i) the damage recognition/incision step by disturbing DNA–protein interactions mediated by magnesium ions and (ii) the polymerization and/or ligation step, since DNA polymerases as well as DNA ligases depend on Mg(II) as a cofactor. When investigating the DNA polymerization on synthetic polynucleotides, Ni(II), but also Co(II) were able to substitute for Mg(II), thereby

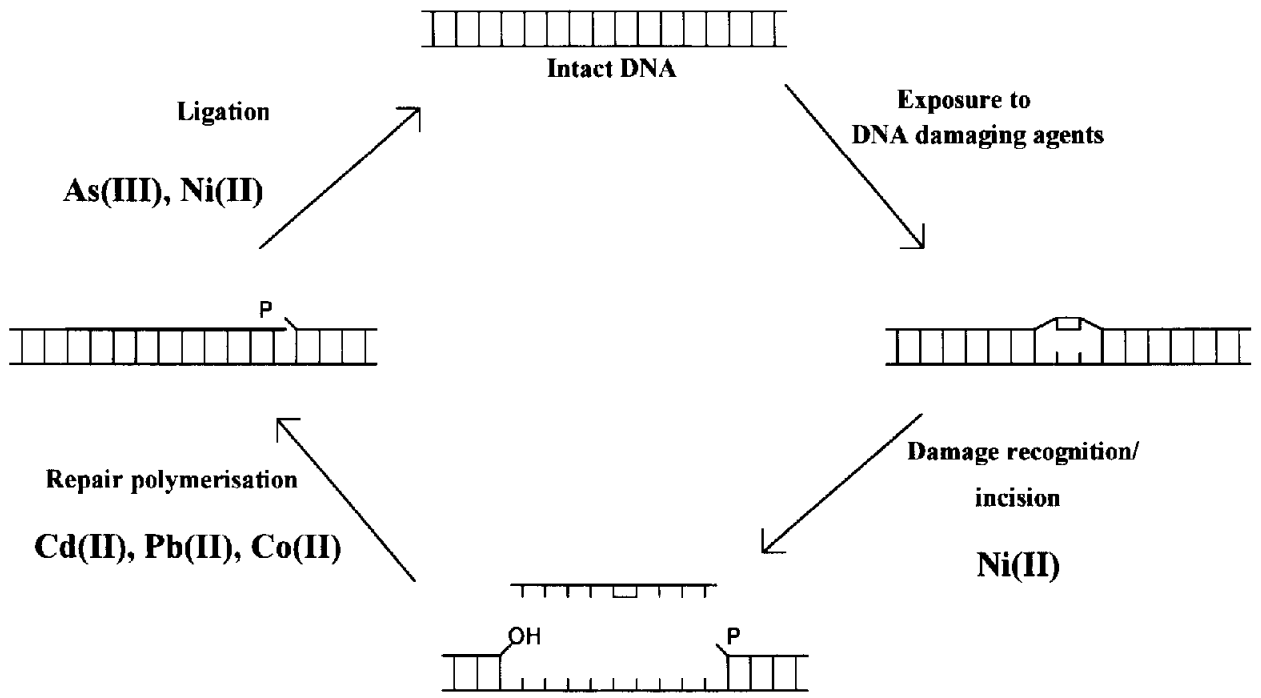

Figure 1. Inhibition of nucleotide excision repair by carcinogenic metal ions in mammalian cells: proposed sites of action.

partly activating polymerases derived from different sources but decreasing their fidelity (Sirover & Loeb 1976, 1977). A rather complex interference with DNA replication by Ni(II) has also been observed in more recent studies: While the incorporation of DNA precursors *in vitro* into activated calf thymus DNA was inhibited at all concentrations of Ni(II) when applying purified HeLa DNA polymerase α, in intact HeLa cells the rate of DNA replication was stimulated at low and inhibited at high concentrations of Ni(II) (Chin *et al.* 1994). In addition to the potential substitution for Mg(II), the interaction with zinc might also be relevant: in several *in vitro* studies, Ni(II) was able to displace zinc from 'zinc finger' structures, e.g. in the human transcription factor SP1 (Nagaoka *et al.* 1993). Similarly, Cd(II), Co(II) and Ni(II) have been shown to bind to zinc finger residues in the bovine estrogen receptor, with Cd(II) and Co(II) restoring its DNA binding activity (Predki & Sarkar 1992). Since the DNA binding of at least one eucaryotic repair protein involved in damage recognition, XPAC, is mediated by zinc finger structures (Tanaka *et al.* 1990), this competition might also be relevant for the disruption of repair processes by metal ions. Finally, the initiation of repair events depend on calcium-regulated processes: when investigating specific interactions of cell free extracts derived from HeLa cells with UV-irradiated DNA, the specific binding of a protein to UV-damaged DNA was diminished in cells treated with the calcium ionophore A 23187 (Chao & Huang 1993). Since cadmium ions have been shown to interfere with the calcium-dependent signal transduction (Beyersmann *et al.* 1992), this interaction might also be important.

Conclusions and perspectives

The current knowledge on mechanisms in metal genotoxicity suggests that there is no common mechanism which could account for the carcinogenic potential of the diverse metals and their compounds. The genotoxic potential depends largely on the bioavailability of the actual species. Two modes of action seem to be predominant: the enhanced formation of reactive oxygen and other radical species, leading to oxidative DNA damage, most clearly demonstrated for chromium compounds, and the interference with DNA repair and/or DNA replication processes, which are very sensitive towards the action of arsenic, nickel, cobalt, lead and cadmium. However, the underlying mechanisms are diverse, and depend on the ability of the specific metal compounds to compete with essential metal ions and to form complexes with biomolecules. Furthermore, it has to be kept in mind that epigenetic mechanisms like an interference with processes involved in tumor promotion might also contribute to the carcinogenic potential of metal compounds, e.g. by a disruption of calcium-mediated cellular signal transduction pathways. Taken together, future research should focus on the question which cellular processes are most sensitive towards the action of the respective metals in order to assess its relevance for the carcinogenic potential.

Acknowledgments

This work was supported by grant no. Be 561/6-1 of the Deutsche Forschungsgemeinschaft and by the University of Bremen.

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